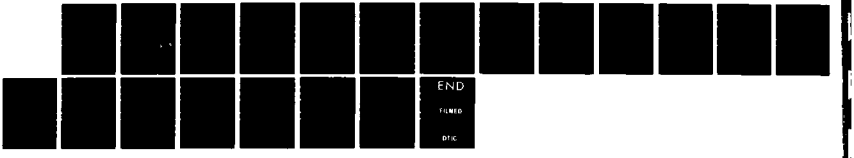
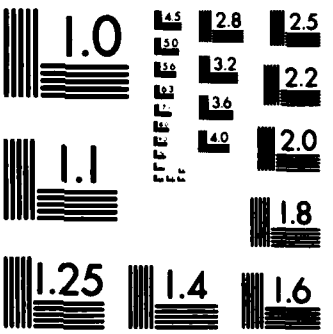
AD-A150 715 NONSPECIFIC RESISTANCE INDUCED BY AN IMMUNOPHARMACOLOGIC AGENT DERIVED FR..(U) ALABAMA UNIV UNIVERSITY DEPT OF MICROBIOLOGY A L WINTERS ET AL. 1/1

UNCLASSIFIED 31 JAN 85 N00014-83-K-0597 F/G 6/5 NL





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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
TITLE (and Subtitle) NONSPECIFIC RESISTANCE INDUCED BY AN IMMUNOPHARMACOLOGIC AGENT DERIVED FROM *BORDETELLA PERTUSSIS*		5. TYPE OF REPORT & PERIOD COVERED Annual: 8/16/83-8/15/84 6. PERFORMING ORG. REPORT NUMBER
AUTHOR(s) Alvin L. Winters, Ph.D., Paul A. LeBlanc, Ph.D. and Gary S. Sloan, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) N00014-83-K-0597
PERFORMING ORGANIZATION NAME AND ADDRESS Department of Microbiology, Box BM The University of Alabama University, AL 35486		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
CONTROLLING OFFICE NAME AND ADDRESS Jeanine A. Majde, Ph.D., Scientific Officer Immunology Code 441, Cellular Biosystems Group Dept. of the Navy, ONR, Arlington, VA 22217		12. REPORT DATE 1/31/85 13. NUMBER OF PAGES 14
MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Unlimited

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

antiviral agent, *Bordetella pertussis*, mutants, virulence factors, pertussigen, immunomodulation, adenovirus, lipopolysaccharide, polysaccharide, vesicular stomatitis virus, endotoxin, endotoxin protein, peritoneal exudate cells, virus migration, splenomegaly, hypothermia, neutralizing antibodies

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Treatment of mice with *Bordetella pertussis* vaccine resulted in resistance to mouse adenovirus infection. Antiviral activity was associated with surface components of *B. pertussis*. Acellular fractions with antiviral activity were obtained by relatively gentle extraction methods, i.e., 1.0 M NaCl extraction or Waring blender treatment. *B. pertussis* lipopolysaccharide extracted by the Westphal procedure possessed antiviral activity. Lipopolysaccharide complexed with protein appeared to have more activity than purified lipopolysaccharide. *B. pertussis* vaccine did not induce

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cross-reacting antibodies with either neutralizing or protective activity toward mouse adenovirus. Peritoneal exudate cells obtained from mice treated with *B. pertussis* vaccine inhibited vesicular stomatitis virus multiplication in L-929 cells. Removal of adherent cells from the peritoneal exudate cell population increased the antiviral activity toward vesicular stomatitis virus.

Mouse adenovirus migrates from the peritoneum to the blood within minutes after intraperitoneal inoculation. Infection with sublethal doses of mouse adenovirus resulted in a transient splenomegaly. Mice developed maximal neutralizing antibody titers after infection with as little as 10 plaque-forming units. Infection with lethal doses of mouse adenovirus resulted in a fever response (hypothermia) before death.

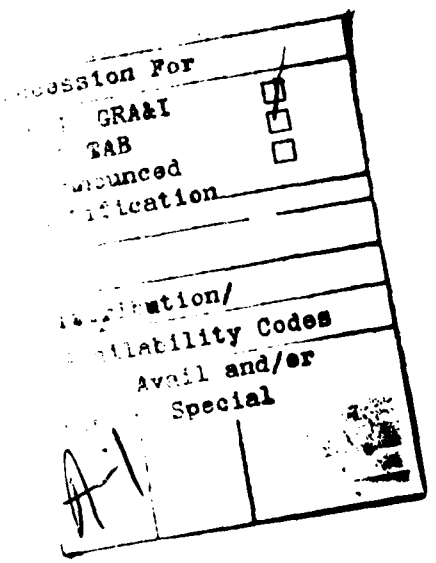

Table 1. Characteristics of *Bordetella pertussis* Vaccines

Strain	Dry Weight (ug)	Agglut.[1]	Protein[2] (ug)	"Glucose"[3] (ug)	Mortality[4] (deaths/total)
18323	166	+	35	2.8	0/5
Tohama I	161	+	40	11.6	0/5
BP347	169	+	41	9.7	0/5
BP359	164	+	44	9.1	0/5
Tohama III	250	+	150	---	0/5
11615	250	-	143	---	0/10
Controls:					
Connaught #1	625	---	100	32.8	0/5
Connaught #2	563	---	106	30.0	0/5
Connaught #3	438	---	97	32.8	0/5

[1]Agglutination with a 1:5 dilution of rabbit antiserum raised against Connaught BPV.

[2]Protein determined by the method of Lowry and coworkers.

[3]Glucose equivalents measured by the anthrone reaction.

[4]Challenged with 4 LD_{50} of MAd1pt4 seven days after treatment.

2.2 BIOCHEMICAL EXTRACTION OF BPV. Dr. Robert Lennon, Connaught Laboratories, Inc., Swiftwater, PA, developed several acellular fractions of *B. pertussis* for possible use as an alternative to the currently used whole cell vaccine with all of its associated side-effects. We have analyzed these fractions for their antiviral activity and have examined a polysaccharide and a lipopolysaccharide fraction of BPV also.

Acellular fraction 15A-1B was obtained from 4 day growth of phase I *B. pertussis* (Connaught Laboratories, Inc., vaccine strain) on Cohen-Wheeler agar. The cells were extracted with 1.0 M NaCl containing 0.05 M sodium phosphate buffer (pH 7.2) and 0.02% thimerosal for 4 days at 4°C. The cells were sedimented and the supernatant decanted. A fractional precipitate (20 to 40% ammonium sulfate saturation) was made, dissolved in buffered saline, dialyzed, and designated fraction 15A-1B. A portion of 15A-1B was treated with 1.0% (v/v) Emulphogene BC 720 (General Aniline & Film Corp., New York, NY). The mixture was incubated for 60 min at 4°C and the precipitate was sedimented at 100,000 x *g* for 60 min. The pellet was resuspended in phosphate buffered saline and the suspension was adsorbed to 1.0% aluminum hydroxide gel. This alum-stabilized preparation was designated as fraction 15A-108A.

Purified LPS was provided by Dr. Thomas W. Klein, University of South Florida, Tampa, FL. The LPS was extracted from *B. pertussis*, strain 3779 BL_2S_4 by a modified Westphal phenol-water procedure. LPS

1.0.0 BACKGROUND. Bordetella pertussis vaccine (BPV) is administered to a large portion of the population in the United States. Our previous observations indicated that BPV has immunomodulatory activity and induces a resistant state to mouse adenovirus, a natural pathogen of mice. Infection with our strain of mouse adenovirus, designated MAd1pt4, results in a systemic infection that culminates in interstitial pneumonia and death with hemorrhagic lungs observed on necropsy. Kirchner and coworkers have reported a similar BPV-induced resistance against herpes simplex virus infection. Acellular extracts of B. pertussis produced by gentle extraction of whole cells with 1.0 M NaCl induced a resistant state to MAd1pt4 also. Thus, the isolation of an immunomodulatory, antiviral agent from B. pertussis for possible human use appears to be a feasible project.

The efforts of the first year of the contract have been focused on identifying the BPV component that is responsible for the antiviral activity of the vaccine. Our preliminary findings indicate the activity resides in lipopolysaccharide (LPS) or a lipopolysaccharide-protein complex.

2.0 IDENTIFICATION OF THE BPV COMPONENT WITH ANTIVIRAL ACTIVITY. Identification of the antiviral component of B. pertussis has been approached by both genetic analysis and biochemical extraction. Antiviral activity was assayed by administering BPV or an acellular component of BPV to mice by the intraperitoneal route and then challenging the animal with a lethal dose of mouse adenovirus seven days later.

2.1 GENETIC ASPECTS OF BPV . Vaccines were developed from several strains of B. pertussis. The organisms were maintained on BG agar base (Difco Laboratories, Detroit, MI) supplemented with 17% defibrinated sheep blood. Vaccines were prepared by harvesting 4-day growth from Cohen-Wheeler agar in phosphate buffered saline (pH7.2) and inactivating the cells by heating (56°C for 30 min) in the presence of 0.02% thimerosal. The vaccines were adjusted to an estimated 4.0 mg (dry weight) per ml in saline-thimerosal diluent and stored at 4°C. Uninoculated Cohen-Wheeler medium did not have antiviral activity.

The parent B. pertussis strain used to develop the Connaught Laboratories' BPV was ATCC 9797, a strain derived from 18323. Antiviral activity was observed with both strains of the same lineage (Table 1). BPV made with strain Tohama I, which was isolated independently of Strain 18323 was protective also (Table 1). In addition, the "virulence factors" of B. pertussis did not play a role in the antiviral activity. Strains Tohama III and 11615 that do not express the virulence factors had protective activity (Table 1). It was interesting to note that strain 11615 did not agglutinate with rabbit antiserum. This strain is thought to be a deep rough strain with a truncated lipopolysaccharide molecule in the outer membrane of B. pertussis (Dr. Mark Peppler, University of Alberta; personal communication). In addition, we have obtained mutants of strain Tohama I (BP347 and BP349) from Dr. Allison Weiss and Stanley Falkow (Stanford University, Stanford, CA). These strains were created by single site insertion of a transposon that abrogated the "virulence factors" ; however BPV made from these strains exhibited antiviral activity (Table 1).

extracted by the Westphal procedure from microorganisms other than B. pertussis were obtained from Sigma Chemical Company, Inc., St. Louis, MO.

Cell surface polysaccharide was harvested and partially purified by the method of Conrad. Four day growth of phase I B. pertussis, strain 18323, was harvested from Cohen-Wheeler agar medium using 0.01 M potassium phosphate buffer (pH 7.0). The capsule (slime) polysaccharide was removed by brief shearing treatment in a Waring blender set at top speed for 45 sec. The cells were removed by centrifugation, the pH of the supernatant was adjusted (pH 2.0) with 6.0 N H_2SO_4 and the polysaccharide was precipitated with acetone. The precipitate was collected by centrifugation, resuspended in water (pH 9.0), and re-precipitated at pH 3.0. The supernatant was dialyzed and concentrated in a flash evaporator.

Antiviral activity was detected in several acellular fractions derived from B. pertussis (Table 2). The standard dose of BPV (250 ug dry weight) contained approximately tenfold the concentration necessary to protect 50% of the test population. Fraction 15A-1B revealed at least 13 proteins ranging in size from 16.5K to 139K upon polyacrylamide gel electrophoresis. The gel pattern was similar to the pattern of outer membrane proteins isolated by Dobrogosz and coworkers. Fraction 15A-108A treated to remove LPS was markedly less active in stimulating lymphocytosis, histamine sensitization, induction of peritoneal exudate cells, and splenomegaly; however, the fraction retained antiviral activity. Partially purified polysaccharide obtained by relatively gentle shearing of the cells possessed antiviral activity. Purified LPS exhibited antiviral activity also. Our preliminary data indicated that the 20 ug (dry weight) dose of LPS was near the minimum protective dose.

The LPS of B. pertussis was unique in its antiviral activity (Table 3). Purified LPS of four different gram-negative species did not have antiviral activity. An antiviral activity of B. pertussis-derived LPS and derivatives has been reported previously by LeDur, Chaby, Szabo and coworkers. The protective activity of LPS observed by LeDur and coworkers developed within 24 h. We may be examining the same activity; however, the duration of our antiviral effect (5 to 35 days) would suggest that we are observing a different component of immunomodulation.

Additional evidence for a polysaccharide moiety being involved in the antiviral activity is the observation that the vaccine can be inactivated by periodate treatment. Treatment of BPV with 100 mM periodate for 48 h destroyed the protective activity (Table 4). Milder periodate treatment as described by Tuomanen and Hendley to inactivate B. pertussis glycocaylx adherence to bronchial ciliated epithelial cells did not abrogate the protective activity.

Taken together, the data indicate that LPS is probably involved in the BPV-induced immunomodulation that results in resistance to MAd1pt4. However, when one examines the relative specific activity of B. pertussis LPS in the various acellular fractions, one observes that LPS in conjunction with native protein appears to be more effective, e.g., BPV (25 ug dry weight) and 15A-108A versus purified LPS (20 ug dry weight), which was near the minimum effective dose. Sultzer, Craig, and coworkers have observed that the endotoxin-associated proteins of B. pertussis are unique in their adjuvant activity. Their findings also suggest that LPS complexed with endotoxin-associated proteins might have increased antiviral activity. We are currently focusing our efforts on the isolation of an LPS-protein complex from B. pertussis.

Table 2. Activities of *Bordetella pertussis* Vaccine and Acellular Extracts

Treatment (i.p.)	Dry Weight (ug)	Protein (ug)	LPS[1,2] (ug)	Biologic Activity	Mortality[3] (deaths/total)
BPV, Connaught	250	51	2.34	LPF, HSF, PEC-stimulation, splenomegaly	0/21
BPV, Connaught	25	5	0.23	---	19/47
15A-1B, Connaught	---	46	147	LPF, HSF, decreased splenomegaly	3/16
15-108A, Connaught	---	12	.039	decreased LPF,HSF, and PEC stimulation, splenomegaly not detected	4/20
Purified LPS, 3779	20	<0.2	40	---	0/5
Polysacch, 18323	415	11.5	100	---	0/5
Diluent	---	---	---	---	21/21

[1] The efficiency of detection was assumed to be similar for free and bound LPS as reported by Jorgesen and Smith.

[2] Amebocyte lysate assay, Sigma Chemical Company, St. Louis, MO.

[3] Challenged with 2-10 x 10^7 PFU MAd1*pt*4 seven days after treatment.

3.0 MECHANISM OF BPV-INDUCED ANTIVIRAL ACTIVITY. In anticipation of isolation of a purified component with antiviral activity, preliminary studies of possible mechanisms of immunomodulation were carried out.

3.1 INDUCTION OF A HUMORAL RESPONSE.

The serum of BPV-treated mice did not contain crossreacting antibody that would neutralize MAd1*pt*4 *in vitro*. However, several published reports indicated that certain monoclonal antibodies do not neutralize viruses, but will protect a test animal against a challenging virus infection. Serum was obtained from mice treated with BPV for 21 days. Passive immunization against MAd1*pt*4 infection was attempted by inoculating 0.5 ml of the serum into each animal 12 h prior to virus challenge. No protection was observed.

Table 3. Activity of Purified Lipopolysaccharides[1]

Treatment, (20 ug)	Mortality[2] (deaths/total)
Escherichia coli, Serotype 055:B5	5/5
Vibrio cholerae, Serotype Inaba 569B	5/5
Salmonella typhimurium	5/5
Salmonella minnesota	5/5
Bordetella pertussis, strain 3779[3]	0/5

[1] Purified by the Westphal phenol-water extraction method.

[2] Challenged with 2 x 10^7 PFU MAdlpt4 seven days after treatment.

[3] Provided by Dr. Thomas W. Klein, University of South Florida, Tampa, FL.

Table 4. Modification of BPV

Reaction Conditions Prior to Animal Injection	Mortality[1] (deaths/total)
BPV (no treatment)	0/5
BPV treated with 0.1 M $NaIO_4$ for 48 h at 25°C.	5/5
BPV treated with 0.5 mM $NaIO_4$ for 30 min at 25°C.	0/5

[1] Challenged with 4 LD_{50} of MAdlpt4 seven days after treatment.

3.2 INDUCTION OF A CELLULAR RESPONSE. Peritoneal exudate cells (PECs) were chosen for study because of their direct exposure to BPV during treatment, ease of harvest, and the prior report by Morahan and coworkers of antiviral activity of PECs following immunomodulation. Vesicular stomatitis virus (VSV) infection was chosen for the initial development of an in vitro assay of antiviral activity due to the ease and rapidity of the VSV plaque assay; one day as compared to 12-14 days for MAdlpt4. Mice were inoculated i.p. with BPV or vaccine diluent

(saline-merthiolate;SM) and seven days later peritoneal lavage was performed to harvest PECs. Cells were cultured in microtiter plates or 35 mm culture dishes. The following cell concentrations were added to each microtiter well, singularly or in combination:

L929 - 4.0×10^3 cells
SM-PECs - 1.0×10^5 cells
BPV-PECs - 1.0×10^5 cells

The cell culture systems were infected by adsorbing 20 PFU of VSV, serotype Indiana, to L-929 cells for 2.0 h. This strain of VSV did not produce a lethal infection in mice with 10^8 PFU or less. Twelve microtiter cultures were pooled for each point assayed. The growth cycle of the virus appeared to be essentially complete within 60 h of infection in L-929 cell cultures (Figure 1). Combination cultures using the above effector cell (PEC) concentration to obtain an effector to target cell ratios of 25:1 were constructed also. Virus was adsorbed to L-929 cell cultures (calculated MOI of 1.0 PFU per 200 cells) for 2.0 h to allow entry of the virus into the permissive cell and then effector cells (either SM-PECs or BPV-PECs) were added. As before, 12 microtiter wells were pooled for each assay of infectivity. Effector cells harvested from SM-treated mice had no observable effect when cocultured with VSV-infected L-929 cells, whereas effector cells from BPV-treated mice decreased the ability of L-929 cell cultures to synthesize virus by approximately 90% (Figure 1). This observation was confirmed by statistical analysis of titers obtained from ten individual pools of microtiter well cultures 60 h after infection (Table 5).

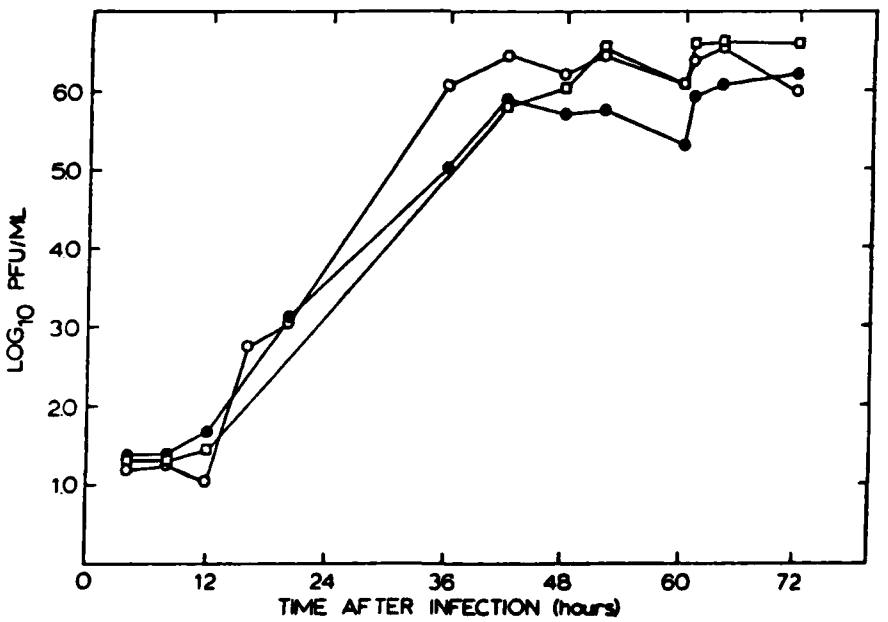

Figure 1. Growth Curve of Vesicular Stomatitis Virus in L-929 Cell and Peritoneal Exudate Cell Cocultures. Vesicular stomatitis virus was adsorbed to cultures of L-929 cells. After virus adsorption either no cells (□), SM-induced PECs (○), or BPV-induced PECs (●) were added and virus infectivity was assayed on L-929 cells thereafter.

Table 5. Activity of BPV-induced Peritoneal Exudate Cells on Vesicular Stomatitis Virus Synthesis in L-929 Cells

Treatment of Infected Cultures	Virus Synthesized 60 h After Infection (PFU x 10^{-6})	
	Mean	Standard Deviation
Control (no PEC addition)	18.4[a],[c]	7.8
SM-PEC coculture	37.1[a],[b]	28.8
BPV-PEC coculture	2.6[b],[c]	0.9

[a] Student's t-test, d.f. = 18, P = .059.
[b] Student's t-test, d.f. = 18, P = .001
[c] Student's t-test, d.f. = 18, P < .001

Although there were many more cells in the PEC population, as compared to the L-929 cell population in the cultures, the PECs were notably less permissive (Figure 2). In addition, the BPV-induced PECs were less permissive than the SM-induced PEC cultures. Thus, the PEC fraction in a combination culture of PECs and L-929 cells would contribute less than one percent of the progeny virus.

A portion of the inhibition of virus synthesis was due to interaction of the BPV-induced PECs and the cells infected initially. Virus was adsorbed to L-929 cell cultures (calculated MOI of 1.0 PFU per 200 cells) for 1.0 h to allow entry of the virus into the permissive cell. The infection was carried out in 35 mm culture dishes with the same density of L-929 cells per unit area as was used in the microtiter wells. Effector cells (either SM-PECs or BPV-PECs) were added and the combination culture was incubated an additional 2.0 h. Excess L-929 cells at a concentration that would form a monolayer within 24 h were then added and 1.0 h later an agar-based overlay medium was added to localize virus cytopathology for visualization of infectious centers or plaques. Approximately half of the infectious centers were inactivated by BPV-induced PECs (Table 6). Virus infection cycles in L-929 cells subsequent to the initial round of multiplication were probably inhibited also.

The activity of BPV-induced PECs was specific and was directed toward virus-infected L-929 target cells and not toward both uninfected and infected cells. L-929 cells were planted at a concentration of 40 cells per 30 mm dish and cultured 24 h. BPV-induced or SM-induced PECs were added to the L-929 cell cultures at the previous effector cell to target cell ratio. The cells were then maintained in coculture for 6 days and the colonies of L-929 cells counted.

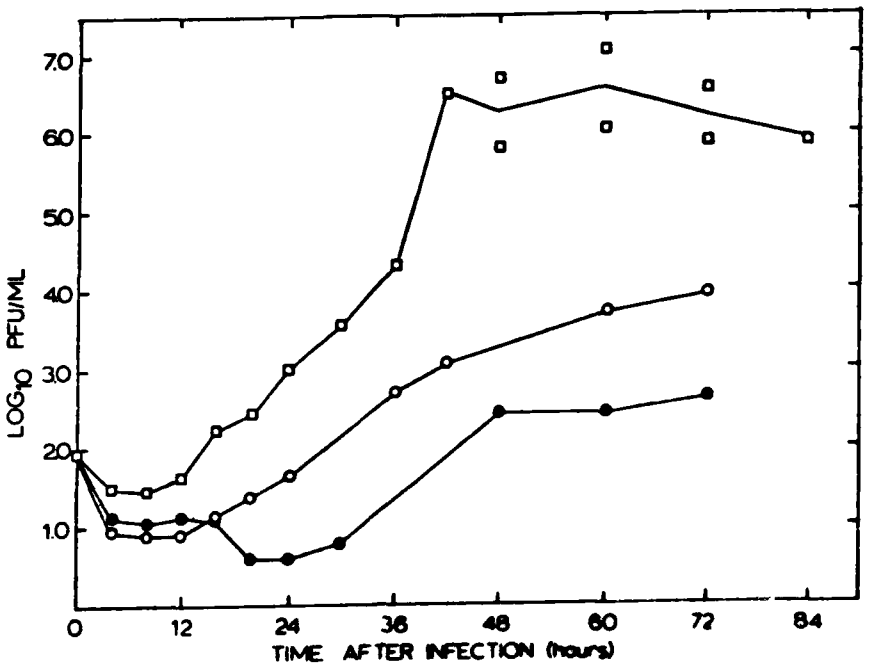

Figure 2. Growth Curve of Vesicular Stomatitis Virus in L-929 Cell and Peritoneal Exudate Cells. Vesicular stomatitis virus was adsorbed to cultures of L-929 cells (□), SM-induced PECs (○), or BPV-induced PECs (●) and virus infectivity was assayed on L-929 cells thereafter.

Table 6. Activity of BPV-induced Peritoneal Exudate Cells on Vesicular Stomatitis Virus Infectious Centers

Treatment of Infected Cells	Infectious Centers in L-929 Culture	
	Mean	Standard Deviation
Control (no PEC addition)	62.8[a,c]	8.6
SM-PEC coculture	77.4[a,b]	11.0
BPV-PEC coculture	53.0[b,c]	6.4

[a] Student's t-test, d.f. = 18, P = .004.
[b] Student's t-test, d.f. = 18, P < .001.
[c] Student's t-test, d.f. = 18, P = .009.

BPV-induced PECs exhibited some toxicity toward uninfected L-929 cells; however, the amount of colony formation indicated that nonspecific toxicity did not play a major role in the antiviral activity of BPV-induced PECs (Table 7). Coculturing of SM-induced PECs with L-929 cells markedly decreased the number of microcolonies that developed. This decrease did not appear to be due to toxicity. The coculture of SM-induced PECs and L-929 cells resulted in a marked increase of L-929 cell migration or spreading and enumeration of discrete colonies was difficult.

An initial characterization of the PEC population with antiviral activity was carried out. Infected L-929 cells were cocultured with either PECs or PECs minus the adherent cell population. SM-induced and BPV-induced PECs were harvest as described previously. A portion of each cell suspension was planted in 60 mm cell culture petri dishes and incubated 2.0 h. Nonadherent cells were removed by washing and adjusted to the same concentration as the total PEC population. Approximately 50% of the total population was adherent cells, consequently the nonadherent cell population was enriched approximately twofold.

Table 7. Colony Formation in Cocultures of L-929 Cells and Peritoneal Exudate Cells

Treatment	Colonies after 6 Days of Culture	
	Mean	Standard Deviation
Control (no PECs added)	30.3[a],[c]	4.9
SM-PEC coculture	9.3[a],[b]	2.8
BPV-PEC coculture	23.6[a],[b]	4.1

[a] Student's t-test, d.f. = 18, $p < .001$.
[b] Student's t-test, d.f. = 18, $p < .001$.
[c] Student's t-test, d.f. = 18, $p = .003$.

Removal of the adherent cell population increased the antiviral activity of BPV-induced PECs (Table 8). Either the cell population responsible for the antiviral activity was enriched by the procedure or the adherent cell population in the original PEC suspension was inhibiting the antiviral activity of that suspension.

Taken together, our data indicate that BPV-induced PECs exhibit an antiviral activity toward VSV infected L-929 cell; however, the system does not provide an easily assayed expression of the antiviral activity, i.e., a relative large number of cultures and statistical analysis would be necessary to evaluate a given acellular fraction of B. pertussis. Our future efforts will be directed toward using herpes simplex virus infected cells as target cells.

4.0 PATHOGENESIS OF HAd1pt4. Studies of HAd1pt4 pathogenesis were extended to aid in the interpretation of the model used for screening antiviral activity of B. pertussis.

4.1 MIGRATION OF THE INOCULUM. The kinetics of MAd1pt4 migration from the peritoneal cavity after inoculation was examined. Mice were injected i.p. with 10^4 PFU (sublethal dose) and then a pocket bleed and peritoneal lavage were performed. Infectious virus was assayed in each system at various times after infection. Unexpectedly the virus appeared in the plasma very soon after injection into the peritoneum (Table 9). Most of the virus had migrated out of the peritoneum by 3.0 h. The virus exhibited very low titers in the circulating blood by 24 h and then increased after 3 to 4 days, probably due to active replication. Significant changes in the number of cells or cell types in the peritoneum during this period were not observed.

4.2 SYSTEMIC INFLAMMATION. Infection with a sublethal dose of MAd1pt4 resulted in a significant, transient splenomegaly after infection (Figure 3). The increased spleen size was due in part to an increased number of cells in the spleen. Infection with a sublethal dose of MAd1pt4 did not induce a fever response of hypothermia characteristic of mice (Figure 4). In contrast, lethal dose infection induced a fever response at least two days before death (Figure 5).

Table 8. Activity of Nonadherent BPV-induced Peritoneal Exudate Cells on Vesicular Stomatitis Virus Synthesis in L-929 Cells

Treatment	Virus Titer after 60 h (PFU x 10^{-6})	
	Mean	Standard Deviation
Control (no PEC addition)	10.6	3.8
SM-PEC coculture	3.5[a]	1.5
SM-PEC (minus adherent cells) coculture	1.8[a]	1.3
BPV-PEC coculture	2.5[b]	0.6
BPV-PEC (minus adherent cells) coculture	1.3[b]	0.4

[a] Student's t-test, d.f. = 8, p = .083
[b] Student's t-test, d.f. = 8, p = .004

Table 9. Virus Migration from the Peritoneal Cavity to the Bloodstream[1]

Time After Infection (h)	Peritoneal Cavity (PFU)	Plasma[2] (PFU)
0.08	19,800	308
0.25	7,300	396
0.42	9,500	484
1.0	5,000	1,100
3.0	280	308
6.0	920	2,442
12	20	110
24	10	44
48	120	44
72	60	968
96	140	550
168	90	220

1 Mice were injected i.p. with 10^4 PFU.

2 Based on a total plasma volume of 1.10 ml as reported by Friedman.

4.3 HUMORAL RESPONSE. Mice respond to sublethal dose infection with detectable neutralizing antibodies within 14 days after infection (Table 10). As little as 10 PFU will cause seroconversion of the mouse (Table 11). These data reinforce the extreme caution one must use in working with MAd1pt4 and thereby prevent possible communication between the virus laboratory and the animal facility.
The role of interferon in mouse adenovirus infection is questionable at this time. We have repeatedly fail to demonstrate significant increases in levels of interferon in the blood or peritoneal lavage after virus infection. In addition, reproducible increases of interferon after BPV treatment were not observed. Numerous attempts to induce interferon in L-929 cells by MAd1pt4 infection have failed. Finally, the sensitivity of MAd1pt4 to the effects of interferon was fourfold less than VSV.

5.0 PUBLICATIONS FROM THIS CONTRACT.

Winters, A. L., D. W. Baggett, W. R. Benjamin, H. K. Brown, and T. W. Klein. 1985. Resistance to adenovirus infection after administration of Bordetella pertussis vaccine in mice. Infect. Immun. 47: (in press).

Winters, A. L., D. W. Baggett, J. D. Lee, G. L. Sloan, R. D. Lennon, and R. S. Stinson. 1985. Immunomodulation by Bordetella pertussis: Antiviral effects. In C. R. Manclark and W. Hennessen (eds). Proceedings of the Fourth International Symposium on Pertussis. S. Karger, N.Y., (in press).

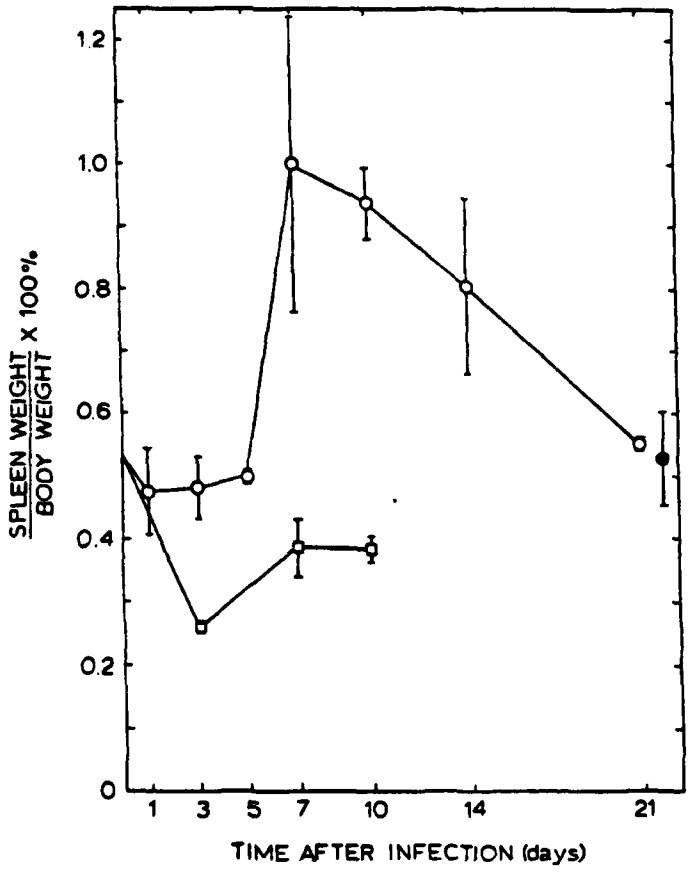

Figure 3. Splenomegaly Following MAd1pt4 Infection. Mice were inoculated i.p. with 10^4 PFU (○) or L-929 cell extract control (□). Uninjected mice were used as control also (●). Points represent the average splenic index of 3 to 15 mice.

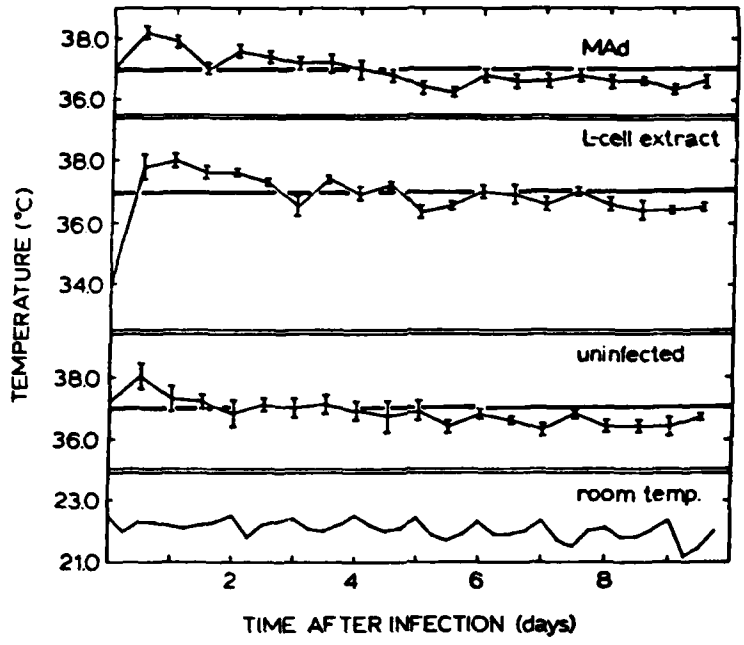

Figure 4. Temperature of Mice Following MAd1pt4 Infection with a Sublethal Dose. Mice were inoculated i.p. with 10^4 PFU. Rectal temperatures were obtained at 12 h intervals thereafter. Each point represents the mean temperature of five mice. The solid bar references 37°C.

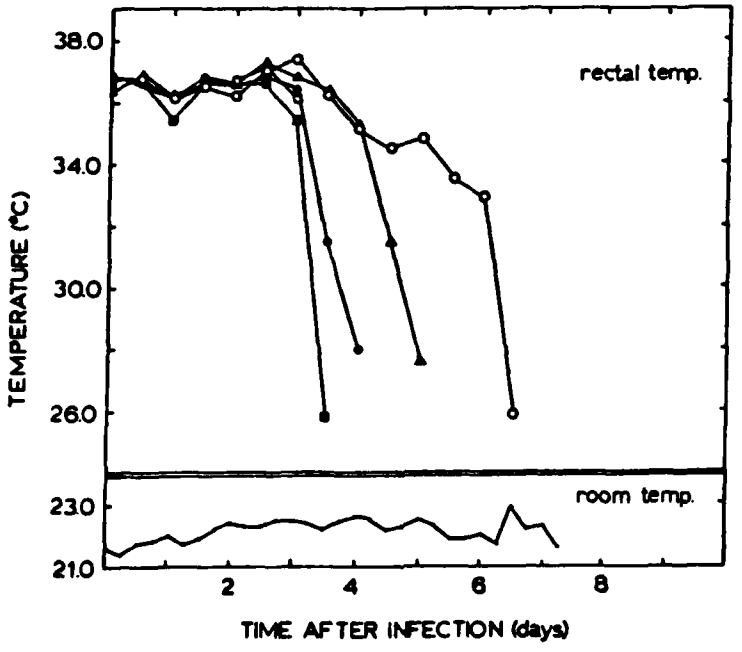

Figure 5. Temperature of Mice Following MAd1pt4 Infection with a Lethal Dose. Mice were inoculated i.p. with 2.8×10^7 PFU. Rectal temperatures were obtained at 12 h intervals thereafter. Each symbol represents an individual mouse.

Table 10. Development of Neutralizing Antibodies After MAd1pt4 Infection[1]

Time of Serum Sample (days)	Titer[2,3]
7	< 10
14	47
21	160
28	213
35	267

[1] Mice were infected by i.p. inoculation with 1.0×10^4 PFU.

[2] One unit was defined as the antibody concentration necessary to neutralize 16 $TCID_{50}$ in an L929 culture in a microtiter well.

[3] Represents the average titer in a group of three mice.

Table 11. Dose-response of MAd1pt4 and Neutralizing Antibodies

Virus Dose (PFU)	Neutralizing Antibody (Titer)[1,2]
0 (L-929 cell extract)	< 10
10^1	213
10^2	133
10^4	160

[1] Represents the average titer in a group of three mice.

[2] Serum obtained 21 days after virus inoculation i.p.

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